Invasive Ascidians in Washington State—Problematic Species and Current Status

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by
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The tasks outlined in this contract, and progress on each of them, are as follows (see p. 14 of contract, attachment B):

1. Analyze biological samples suspected of containing *Didemnum*.

From Sept. 2004 to the present, I have analyzed numerous samples from all over Puget Sound, the Hood Canal and Strait of Juan de Fuca, most of them collected by scuba-diving members of the public who have been made aware of this invasive species as a result of public service workshops sponsored by WDFW and WA Sea Grant. Fortunately most of the samples have NOT been *Didemnum* sp. A; they have been various types of sponges or other species of ascidians. After a full year of analyzing samples, *Didemnum* sp. A is known to be present at

a) Totten Inlet Taylor Shellfish mussel rafts, abundant on the rafts, lines and mussels themselves. No eradication efforts have been made.

b) Edmonds Underwater Park where a large colony or colonies on a sunken boat was eradicated in October/November 2004 but where strict monitoring is ongoing. *Didemnum* sp. A is thus controlled at this site and possibly completely eradicated.

c) Des Moines Marina, where a colony was observed (but not eradicated) in November 2004 on a submerged crab trap tied to a floating dock. No other *Didemnum* sp. A were observed during a thorough search of numerous docks at this marina at that time.

d) Dabob Bay, Hood Canal, Taylor Shellfish mussel lines. No eradication efforts have been made.

2. Develop and conduct a series of 5 workshops to train surveyors on how to identify and collect *Didemnum* samples.

I developed a Powerpoint presentation on *Didemnum* sp. A and other invasive ascidians [*Styela clava*, *Ciona savignyi* and *Botrylloides violaceus*] in Washington state. I gave variations of the presentation were given at the following venues. I provided preserved samples for people to examine. In addition, Simon Geerlofs of WA Sea Grant used my Powerpoint talk to give a number of additional workshops.

a) November 18, 2004, Olympia WA ANS Tunicate Response Advisory Group Meeting. Gordon King of Taylor Shellfish provided live *Didemnum*.

b) March 2, 2005 Marker Buoys Dive Club monthly meeting, Sunset Hill Community Center, Seattle. (the largest turnout they had ever had; over 50 attended)

c) April 14, 2005 Nisqually Wildlife Refuge, WA ANS Committee Meeting

d) May 21, 2005 Frances Anderson Cultural & Leisure Center, Edmonds, sponsored by WA Sea Grant. Live and preserved specimens were provided, and the meeting was followed by a dive at Edmonds Underwater Park organized by Simon Geerlofs.

e) May 22, 2005 Port Townsend Marine Science Center, sponsored by WA Sea Grant and organized by Simon Geerlofs. Many species of live ascidians, native and non-native, were collected prior to the workshop for attendees to examine.

f) Nov. 5, 2005 Pleasant Harbor Marina, Hood Canal, a scuba dive eradication/mitigation effort to remove all *Styela clava* from floating docks and moored boats, co-sponsored by WA Sea Grant. I participated in the pre-dive talk and provided land-based support to the divers.

Additional talks:

a) Keynote speaker at the Intl. Invasive Sea Squirt Conference at Woods Hole Oceanographic Institution, Woods Hole, MA April 21-22, 2005

b) All day workshop at Pacific Biological Station, Nanaimo, British Columbia, Nov. 4, 2005. Many species of live native and non-native ascidians were collected prior to the workshop. Emphasis was on *Didemnum* sp. A and *Styela clava*. The participants (over 40) included govt. employees concerned with fisheries and aquaculture in BC and a number of oyster and mussel farmers for whom fouling by non-native ascidians including *Didemnum* sp. A is a big problem.

3. Assist in DNA analysis of *Didemnum* by supplying, identifying and dissecting samples.

It has been very difficult to find someone to do this analysis, which includes sequencing the mitochondrial cytochrome oxidase I gene from larvae. I have spent many hours isolating brooded larvae from alcohol-preserved *Didemnum* colonies from the U.S. east coast, west coast, British Columbia, New Zealand, and France. During fall 2004 I trained a student in Dr. Billie Swalla's lab at Univ. of Washington Dept. of Biology how to isolate larvae, and personally isolated many for her. No lab work on these was completed, and she switched to a different project. In May 2005 I sent about 10 samples, each with approximately 80 larvae, to Dr. Vicky Webb in New Zealand, a molecular biologist who promised to complete the work but who has not yet done so.
In November 2005 I supplied isolated larvae from a number of samples to another student of Dr. Swalla. He is working diligently but has not yet obtained usable data.
Dr. Swalla is currently beginning a collaboration with Dr. Rusty Rodriguez, Project Leader/Microbiologist, U.S. Geological Survey, Seattle, WA. Rusty is analyzing ballast water and developing species-specific tags for invasive species. He hopes to sequence the ITS region of the DNAs that the Swalla lab (including myself) can supply him, for invasive and local native ascidians in Puget Sound. This is not exactly the goal I have, which is to analyze *Didemnum* sp. A samples from around the world to determine if they are all the same species or not, but it should provide additional information.

4. View 5 hours of underwater video taken by Homeland Fire & Resource Mgement Group to estimate feasibility of using this technology to identify suspected colonies of *Didemnum* in Puget Sound.

The videos were viewed and deemed of much too poor quality, taken at too far away from the benthos and at too fast a tow speed, to be of any use whatever.

Additional tasks completed under this contract:
Besides *Didemnum* sp. A, there are 2 other species of invasive ascidians in Washington state that are of concern to WDFW: *Styela clava* and *Ciona savignyi*. I have mentioned above that I included these 2 spp. in my talks and workshops.

I collected 50 *Styela clava* (with the help of my husband Charles) from Pleasant Harbor marina on Nov. 5 according to a specific collecting protocol of Dr. John Bishop, Marine Biological Assoc., Citadel Hill, Plymouth, UK, who is analyzing the DNA of *S. clava* worldwide to determine paths of invasion. A small sample of branchial tissue was dissected out of each animal, preserved in 95% ethanol in 1.5ml tubes, and the samples were mailed to Dr. Bishop. These will be the first *S. clava* samples from the Pacific NW ever to be subjected to DNA analysis, and should provide valuable new information on this invasive species.
At this time *S. clava* is known from only 2 other locations in Washington: Neah Bay marina and Blaine marina. It is present at numerous locations in southern British Columbia, mostly marinas but also a few oyster farms.

I confirmed the identity of *Ciona savignyi* from geoduck tracts at the south end of Hood Canal, at the mouth of the Tahuya River, collected by Don Rothaus and Bob Sizemore of WDFW. While it is easy for me to distinguish *C. savignyi* from *C. intestinalis* by surface features, the definitive comparison involves a very tedious and difficult dissection of perfectly relaxed and preserved specimens, so Bob supplied me with living animals so that I could preserve them myself. There were no *Ciona* at this site in the 1990's, but now there are thousands. Bob wrote: "We appreciate all

of your work on these critters. In my 15 years of diving with WDFW, this was one of the more unusual and startling observations that we've made."

Ciona savignyi was very abundant at Des Moines Marina in 1998 (Cohen, A., Mills, C., Berry, H., Wonham, M., Bingham, B., Bookheim, B., Carlton, J., Chapman, J., Cordell, J., Harris, L., Klinger, T., Kohn, A., Lambert, C., Lambert, G., Li, K., Secord, D. and Toft, J. 1998. Report of the Puget Sound Expedition Sept. 8-16, 1998; A Rapid Assessment Survey of Non-indigenous Species in the Shallow Waters of Puget Sound. Wash. State Dept. Nat. Res., Olympia, WA. 37 pp.). It was absent from Edmonds Marina but had begun to appear by 1999 and is now very abundant at Edmonds and continues to be abundant at Des Moines. My husband and I survey a number of sites around Puget Sound periodically for invasive ascidians, and have found *C. savignyi* at the Tacoma Yacht Club also.

The colonial ascidians *Botrylloides violaceus* and *Botryllus schlosseri* are non-native foulers common on marina floats and mussel and oyster farms throughout the Pacific NW. They have been in this area for over 40 years and were probably introduced with imported oysters from the east coast and Japan. They are now ubiquitous in this area.

Conclusions and Recommendations

Didemnum sp. A is currently known primarily on Taylor Shellfish mussel lines in south Puget Sound and Dabob Bay. Any efforts to eradicate it should involve this company. In Prince Edward Island, *Botrylloides violaceus* and *Styela clava* are a huge fouling problem on mussel lines and gear. Vinegar (acetic acid) sprays have proven to be a highly successful treatment to kill the ascidians. I recommend that all shellfish farmers be required to spray or dip their mussels and gear periodically to rid them of these 2 spp. plus *Didemnum* sp. A. All shellfish and gear should be required to be sprayed or dipped prior to moving them from one grow site to another, as I recently recommended to Russell Rogers of WDFW, who is responsible for issuing permits for moving of shellfish stock.

The *Styela clava* eradication effort by scuba divers at Pleasant Harbor Marina on Nov. 5 was not successful, because the *S. clava* infestation was much larger than anyone had realized. However, it is still theoretically possible to eradicate *S. clava* because this species is known from only 3 locations so far. It will require money and effort, however. By Nov. 5 the species had completed its breeding season, so it will not be spawning again until late spring or early summer 2006. It is possible that winter rains will lower the surface salinity enough to kill some of the *S. clava*, especially the younger animals. I recommend another survey dive in March to assess the size of the population at Pleasant Harbor, and make a decision at that time as to whether additional eradication dive days are a realistic and feasible method of eradicating this species.

I have received numerous samples, and digital photos via email attachment, of purported *Didemnum* sp. A and *S. clava* to verify the identification, and have spent countless hours this past year writing emails to members of the public and to various persons in the WDFW. I expect this will continue and therefore I am requesting that this contract be renewed for another year, at some level of support for the time I know I will be asked to give to WDFW.